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ORIGINAL ARTICLE

# Spectrophotometric methods for simultaneous determination of Carvedilol and Hydrochlorothiazide in combined dosage form

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**KEYWORDS**

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Absorbance ratio method;
Spectrophotometry

**Abstract** Two simple, precise, rapid and economic spectrophotometric methods have been developed for simultaneous determination of Carvedilol (CV) and Hydrochlorothiazide (HCT) in bulk powder and combined dosage form. Method (I) is based on dual wavelength analysis while Method (II) depends on UV-spectrophotometric determination using Q-analysis (graphical absorbance ratio) method.

In Method (I), two wavelengths were selected for each drug in such a way that the difference in absorbance is zero for the second drug. At wavelengths 238 and 248.8 nm HCT has equal absorbance values, therefore, these two wavelengths have been used to determine CV, on similar basis 266 and 289.4 nm were selected to determine HCT in the combined formulation. Method (II) involves the formation of Q-absorbance equation using the respective absorptivity values at 229.2 nm (isoabsorptive point) and 241 nm ($\lambda_{max}$ of CV). The drugs obey Beer's Lambert's law in the concentration range of 1–10 μg/mL for both CV and HCT (for Method I) and in the range of 1–10 and 2–10 μg/mL for CV and HCT, respectively (for Method II). The accuracy and precision were determined and recovery studies confirmed the accuracy of the developed methods that were carried out following the International Conference on Harmonization (ICH) guidelines. Statistical comparison of the suggested methods with the reported spectrophotometric one using $F$ and $t$ tests showed no significant difference regarding both accuracy and precision.



## 1. Introduction

Hydrochlorothiazide (HCT), Fig. 1, is an official drug in both British and United States Pharmacopoeias (BP, 2007; USP, 2007), it is a thiazide diuretic used to treat mild to moderate hypertension, usually in combination with other antihypertensive agents with different mechanisms of action (Wellington and Faulds, 1998). HCT is chemically designated as (6-chloro-3,4-di hydro-2H-1,2,4-benzothiadiazine-7-sulfon-

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amide1,1-dioxide) (Budavari, 2002). Carvedilol (CV), Fig. 2, is an official drug in British and European Pharmacopoeias (BP, 2007; European Pharmacopoeia, 2001), it is an antagonist of $\alpha^1$ and $\beta^1$, $\beta^2$ membrane adrenoceptors and also a modulator of cardiac electrophysiological properties via interaction with $K^+$ and $Ca^{2+}$ ion channels (Karle et al., 2001; Chen and Shih, 2003; Franciosa et al., 2004). It is chemically designated as 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino]-2-propanol (Budavari, 2002). CV is administered alone or together with antihypertensive, diuretic HCT. Combined therapy of CV and HCT had a significantly greater blood pressure reduction than with the same dosage of the drug alone (Radevski et al., 1999).

Literature survey revealed that two RP-HPLC methods were reported for determination of the binary mixture in tablet dosage form (Sultan, 2008; Haggag et al., 2010). Only one method has been reported for estimation of the studied drugs in combined formulation by first derivative spectrophotometric method (Sultan, 2008). But so far no spectrophotometric method has been reported for simultaneous determination of CV and HCT in combination; hence an attempt has been made to develop simple, sensitive, rapid, precise, accurate and economic methods to analyze the studied drugs simultaneously by two spectrophotometric methods, dual wavelength and Q-analysis methods. The proposed methods have been optimized and validated as per the International Conference on Harmonization (ICH) guidelines ICH, 2005 and were found to comply with the acceptance criteria.

## 2. Experimental

### 2.1. Instruments

Double beam UV–Vis spectrophotometer (Shimadzu, kyoto, Japan), model UV-1601 PC with 1 cm quartz cells, connected IBM compatible computer. The bundled software, UV-PC personal spectroscopy software version 3.7 was used, the spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

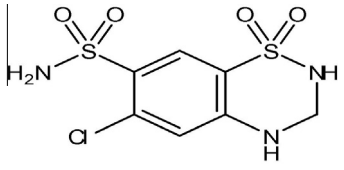


**Figure 1** Chemical structure of HCT.

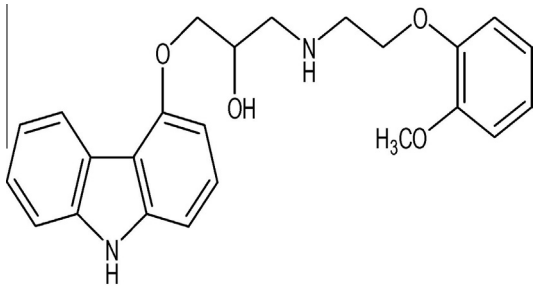


**Figure 2** Chemical structure of CV.

### 2.2. Chemicals and reagents

1. Pharmaceutical grade CV was obtained as a gift by Deltapharma S.A.E., Tenth of Ramadan city, A.R.E. It was certified to contain 98.75% according to the company analysis certificates.
2. Pharmaceutical grade HCT was obtained as gift by Amriya Pharmaceutical Industries, Alexandria, Egypt. It was certified to contain 98.5% according to the manufacturer's method.
3. Methanol and HCl were purchased from (El-NASR Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt). HCl (0.1N) aqueous solution was laboratory prepared.

### 2.3. Marketed formulation

Codilatrol® tablets (Batch No. 070140A), is labeled to contain 25 mg CV and 12.5 mg of HCT, manufactured by Chemipharm Pharmaceutical industries S.A.E. 6th October-Egypt, and purchased from the local market.

### 2.4. Solutions

Standard stock solutions each containing 1000 μg/mL of CV and HCT were prepared separately in methanol. Working standard solutions of these drugs (100 μg/mL) were obtained by dilution of the respective stock solutions in methanol.

## 3. Procedure

### 3.1. Spectral characteristics and wavelength selection

The absorption spectra of 8 μg/mL each of CV, HCT and their 1:1 mixture (containing 4 μg/mL of each) in 0.1N HCl were recorded over the range 200–350 nm using 0.1N HCl as blank. The overlain spectra were observed for selection of the suitable wavelengths for each of the developed methods, Fig. 3.

### 3.2. Linearity

#### 3.2.1. Method I (Dual wavelength method)

Standard solutions of both CV and HCT in the range of 1–10 μg/mL were separately prepared by appropriate dilutions of their respective working standard solutions in 0.1N HCl and then were scanned in the range of 200–350 nm. Absorbance values at both 238 and 248.8 nm (for CV) and at both 266 and 289.4 nm (for HCT) were measured. CV was determined by plotting the difference in absorbance at 238 and 248.8 nm (difference is zero for HCT) against its corresponding concentration. Similarly for determination of HCT, the difference in absorbance at 266 and 289.4 nm (difference is zero for CV) was plotted against the corresponding concentration. The concentrations of the two drugs were calculated each from the corresponding calibration curve equation.

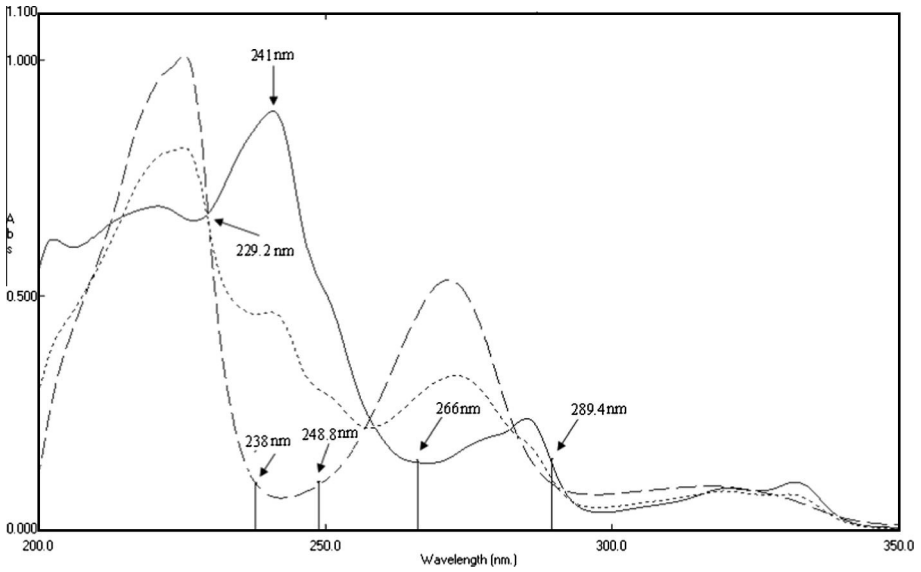


**Figure 3** Zero-order absorption spectra of 8 μg/mL of each of Carvedilol (—), Hydrochlorothiazde (- - -) and 1:1 mixture (……) contains 4 μg/mL of each using 0.1N HCl as a blank.

*3.2.2. Method II (Q-analysis method)*

Standard solutions containing 1–10 μg/mL each of CV and HCT were prepared separately using 0.1N HCl. The absorption spectra of the prepared solutions were recorded in the range of 200–350 nm and the absorbance values at 229.2 nm ($\lambda_{iso}$) and 241 nm ($\lambda_{max}$ of CV) were measured from which the absorptivity values for both drugs at the selected wavelengths were calculated. The method employs Q values and the concentrations of the studied drugs in the prepared solutions were determined by using the following equations:

$$C_x = [Q_m - Q_y/Q_x - Q_y] \times A/A_x$$
$$C_y = [Q_m - Q_x/Q_y - Q_x] \times A/A_y$$

where $C_x$ and $C_y$ are the concentrations of CV and HCT in μg/mL, respectively; $Q_m$ is the absorbance of sample at $\lambda_{229.2}$/absorbance of sample at $\lambda_{241}$; $Q_x$ is the absorptivity of CV at $\lambda_{241}$/absorptivity of CV at $\lambda_{229.2}$; $Q_y$ is the absorptivity of HCT at $\lambda_{241}$/absorptivity of HCT at $\lambda_{229.2}$; $A_x$ is the absorptivity of CV at $\lambda_{229.2}$; $A_y$ is the absorptivity of HCT at $\lambda_{229.2}$; and A is the absorbance of the sample at $\lambda_{229.2}$.

*3.3. Analysis of laboratory prepared mixtures*

Different laboratories prepared mixtures containing different ratios of CV and HCT.. Zero order absorption spectra of these mixtures were recorded using 0.1N HCl as a blank and then the differences in absorbance at 238, 248.8 and at 266, 289.4 nm (for Method I) were measured, also the absorbance values at 229.2 and 241 nm (for Method II) were recorded. From the calculated regression equations, concentrations of CV and HCT in the prepared mixtures were calculated.

*3.4. Analysis of the marketed formulation*

Ten Codilatrol® tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablets powder equivalent to 100 mg CV and 50 mg HCT was transferred into 100-mL calibrated measuring flask, 75 mL methanol was added. The prepared solution was sonicated for 45 mins; the volume was completed with methanol and the solution was then filtered. The filtrate was appropriately diluted with methanol to prepare a working solution equivalent to 0.1 mg/mL CV and 0.05 mg/mL HCT. The prepared working solution was further diluted with 0.1N HCl to get a solution containing 8 μg/mL of CV and 4 μg/mL of HCT. The prepared mixture was analyzed and the absorbance values at the selected wavelengths were determined and the methods given under analysis of laboratory prepared mixtures were followed.

*3.4.1. Recovery studies*

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at different levels (80%, 100% and 120%). Known amounts of the studied drugs were separately added to the pre-analyzed tablets powder and the percentage recoveries were calculated.

## 4. Results and discussion

Development of simple, rapid, sensitive and accurate analytical methods for routine quantitative determination of samples will reduce unnecessary tedious sample preparations, cost materials and laboratories. UV-spectrophotometric methods of analysis offer cost effective and time saving alternative to HPLC method of analysis (Laxman et al., 2010).

As shown in Fig. 3, zero order absorption spectra of CV and HCT show strong spectral overlap which interfere with direct spectrophotometric analysis of the studied drugs without derivatization. On the other hand, the suggested dual wavelength and Q-analysis methods provide a simple, rapid, convenient and accurate way for simultaneous analysis of CV and HCT in their combined dosage form without derivatization procedure.

The main step in the development and validation of an analytical method of analysis is to improve the conditions and

**Table 1** Linear regression and analytical parameters of the proposed methods for determination of Carvedilol and Hydrochlorothiazide.

| Parameters | Dual wavelength method | | Q-analysis method | |
|---|---|---|---|---|
| | CV | HCT | CV | HCT |
| $\lambda$ (nm) | Difference in absorbance between 238 and 248.8 nm | Difference in absorbance between 266 and 289.4 nm | 229.2 nm ($\lambda_{iso}$) and 241 ($\lambda_{max}$) | |
| Beer's law range | 1–10 μg/mL | | 1–10 μg/mL | 2–10 μg/mL |
| Regression equation | Y = 0.0401X + 0.0027 | Y = 0.0449X − 0.0006 | C = (Qm − 0.0805/ 1.3294 − 0.0805) × A/0.085 | C = (Qm − 1.3294/ 0.0805 − 1.3294) × A/0.087 |
| Correlation coefficient | 0.9999 | 0.9998 | – | – |
| Precision | | | | |
| Repeatability | 1.57 | 1.30 | 0.89 | 1.48 |
| Intermediate precision | 0.87 | 1.15 | 1.41 | 0.98 |
| LOD | 0.33 μg/mL | 0.33 μg/mL | 0.20 μg/mL | 0.37 μg/mL |
| LOQ | 1 μg/mL | 1 μg/mL | 0.61 μg/mL | 1.10 μg/mL |

parameters which should be followed in the development and validation (Singh et al., 2011). Different solvents were studied (methanol, ethanol, acetonitrile, water, 0.1N HCl and 0.1N NaOH) to develop suitable methods of analysis, the criteria employed were the sensitivity of the method, availability and toxicity of the solvent. From a solvent effect studies and spectral behaviors of CV and HCT, 0.1N HCl was selected as a solvent for the two suggested methods.

### 4.1. Dual wavelength method

The principle for dual wavelength method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component (Laxman et al., 2010; Davidson et al., 2001; Abdel-Aziz et al., 2002; Zahran et al., 2007; Rajesh et al., 2010a,b). It can be utilized to a great extent without much complication to calculate the unknown concentration of the component of interest in a mixture. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance while the component of interest shows significant difference in absorbance with concentration (Jyotesh et al., 2010).

As per spectra in Fig. 3 we found that absorption values of HCT were the same at 238 and 248.8 nm, so that these wavelengths were selected for the determination of CV. The same as in 266 and 289.4 nm, the absorbance values of CV were the same and hence those two wavelengths were selected for estimation of HCT. Calibration curves for CV and HCT were plotted between the absorbance difference at the selected wavelengths for each drug and the respective concentration. The two drugs obeyed Beer's Lambert's law in the concentration range of 1–10 μg/mL with good correlation coefficients, Table 1. The equations of lines obtained to determine the concentrations of CV and HCT are as follow:

$$A_1 = 0.0401C_{CV} + 0.0027 \quad (1)$$

$$A_2 = 0.0449C_{HCT} - 0.0006 \quad (2)$$

where $A_1$ = the absorbance difference at 238 and 248.8 nm; $A_2$ is the absorbance difference at 266 and 289.4 nm; $C_{CV}$ is the concentration of CV in μg/mL; and $C_{HCT}$ is the concentration of HCT in μg/mL.

### 4.2. Q-analysis (graphical absorbance ratio) method

This method depends on the property that for the substance that obeys Beer's Lambert's law at all wavelengths, the ratio of absorptivity (or absorbance) values at any two wavelengths are constant, independent of the concentration or path length. This ratio is referred as Q-ratio (Bhusari et al., 2009; Wankheda et al., 2009). One of the two selected wavelengths is an isoabsorptive point and the other is the wavelength of maximum absorption of one of the two components (Davidson et al., 2001; Rajesh et al., 2010a,b; Nagulwar and Bhusari, 2010; Devi and Ramakrishna, 2010; Patil et al., 2009).

From the overlain spectra of the two drugs and their mixture, Fig. 3, it is evident that CV and HCT show isoabsorptive points at 229.2 and 317.4 nm, CV has $\lambda_{max}$ at 241 nm while HCT has $\lambda_{max}$ at 271 nm. Using the absorbance values at 229.2 nm ($\lambda_{ios}$) and 241 nm ($\lambda_{max}$ for CV) gave the best results regarding selectivity. The absorbance values at 229.2 and 241 nm for CV in the range of 1–10 μg/mL were obtained and similarly for HCT absorbance values in the range of 2–10 μg/mL were measured, absorptivity coefficients were determined for both drugs and the average values were taken. The values and the absorbance ratio were used to develop the following sets of equations from which the concentration of each component in the sample can be calculated:

$$C_{CV} = (Qm - 0.0805/1.3294 - 0.0805) \times A/0.085 \quad (3)$$

$$C_{HCT} = (Qm - 1.3294/0.0805 - 1.3294) \times A/0.087 \quad (4)$$

where $C_{CV}$ is the concentrations of CV in μg/mL; $C_{HCT}$ is the concentrations of HCT in μg/mL; $Q_m$ is the absorbance of sample at $\lambda_{229.2}$/absorbance of sample at $\lambda_{241}$; and A is the absorbance of the sample at $\lambda_{229.2}$.

To test the selectivity of developing dual wavelength and Q-analysis methods, they were applied for analysis of number of laboratory prepared mixtures containing CV and HCT in different ratios. The good percentage recoveries and low SD values shown in Table 2, confirming the high selectivity of the suggested methods. The proposed methods have been success-

**Table 2** Determination of the studied drugs in the laboratory prepared mixtures (L.P.), pharmaceutical preparations by the proposed methods and statistical comparison with the reported spectrophotometric method.

| Parameters | Dual wavelength method | | Q-analysis method | | Reported method [a] | |
|---|---|---|---|---|---|---|
| | CV | HCT | CV | HCT | CV | HCT |
| Accuracy | 99.96 ± 0.490 | 100.03 ± 1.157 | 99.46 ± 1.485 | 100.22 ± 1.257 | 100.41 ± 0.694 | 99.91 ± 0.961 |
| L.P. mixtures[b] | 100.50 ± 0.915 | 102.00 ± 0.465 | 101.20 ± 1.312 | 100.32 ± 1.093 | | |
| Codilatrol® tablets[c] (B.N. 070140A) | 96.34 ± 0.446 | 92.45 ± 0.777 | 96.46 ± 0.597 | 92.04 ± 0.797 | | |
| Standard addition[b] | 101.89 ± 0.840 | 100.25 ± 1.190 | 102.09 ± 0.744 | 98.11 ± 0.862 | | |
| *F*-test (6.388)[d] | 2.005 | 1.448 | 4.577 | 1.709 | | |
| Student's *t*-test (2.306)[d] | 1.184 | 0.184 | 1.304 | 0.432 | | |

[a] First derivative spectrophotometric determination of HCT at 285 nm and CV at 248 nm using $CH_3OH$ as a solvent.
[b] Average of three determinations.
[c] Average of six determinations.
[d] The values in the parenthesis are the corresponding theoretical values at $p = 0.05$.

fully applied for determination of the studied drugs in bulk powder as well as in their combined dosage form. The results obtained upon using the suggested methods for analysis of CV and HCT in Codilatrol® tablets, Table 2, showed good agreement between the amounts estimated and those claimed by the manufacturer. Moreover, results obtained by the suggested methods showed no significant difference when compared with those obtained by applying the reported spectrophotometric one (Sultan, 2008) as confirmed from *F* and *t* values presented in Table 2. The developing methods have advantages over the reported one on being more simple, rapid, economic and can be used for simultaneous determination of the two studied drugs without derivatization or sample pre-treatment.

### 4.3. Methods validation

Methods validation has been performed as per the International Conference on Harmonization (ICH) guidelines ICH, 2005 and USP requirements (The United States Pharmacopeia, 2007).

#### 4.3.1. Linearity

The linearity of the developed methods was evaluated by analyzing different concentrations of standard solutions of CV and HCT in triplicates. For dual wavelength method, Beer's Lambert's concentration range was found to be 1–10 μg/mL for both CV and HCT. On the other hand, for Q-analysis method the range of CV was found to be 1–10 μg/mL while for HCT was found to be 2–10 μg/mL. The values of correlation coefficients were close to unity indicating good linearity, the characteristic parameters for the constructed equations are summarized in Table 1.

#### 4.3.2. Specificity

The specificity of the proposed methods was assessed by their application to the analysis of laboratory prepared mixtures containing different ratios of intact CV and HCT. Satisfactory results were obtained and presented in Table 2, confirming that each of the cited drugs could be successfully determined without interference from the other.

#### 4.3.3. Accuracy

Accuracy was calculated as the percentage recoveries of blind samples of pure CV and HCT and it indicated the agreement between obtained results and those accepted as true, detailed results are presented in Table 1. To ascertain the accuracy of the suggested methods, recovery studies were carried out by standard addition technique at three different levels (80%, 100% and 120%). Percentage recoveries for CV and HCT by both the two methods were found to be acceptable, Table 2.

#### 4.3.4. Precision

Precision was assessed as RSD% at different levels; *repeatability* was evaluated by the analysis of three different concentrations of pure drugs (3, 6 and 8 μg/mL) for each in triplicates on the same day and *intermediate precision* by repeating analysis of the same concentrations of each seven times on four consecutive days. The results of intra-day and inter-day precision confirmed the precision of the proposed methods, Table 1.

#### 4.3.5. Limits of detection (LOD) and quantitation (LOQ)

They were calculated from the standard deviation ($\delta$) of the response and the slope of the calibration curve ($S$) in accordance to the following equations: LOD = 3.3 ($\delta/S$) and LOQ = 10 ($\delta/S$). Results presented in Table 1, indicated that the method is sensitive for determination of the studied drugs.

## 5. Conclusion

The developed dual wavelength and Q-analysis spectrophotometric methods have been successfully applied for simultaneous determination of CV and HCT in combined sample solution, they were found to be rapid, simple, sensitive and accurate. Once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelengths followed by few simple calculations. The suggested methods were completely validated showing satisfactory data for all the method validation parameters tested. Recovery studies indicated that practically there was no interference from tablet additives, so these methods can be easily and conveniently adopted for routine quality control analysis of CV and HCT.

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